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# 植物内生真菌和稀有放线菌次级代谢产物的研究

**Studies on the Secondary Metabolites of Plant Endophyte  
and Rare Actinomycetes**

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## 常用英文缩写词

缩写式	全称
NMR	Nuclear Magnetic Resonance
ESI-MS	Electrospray Ionization Mass Spectrometry
UV	Ultraviolet
IR	Infra-Red
DEPT	Distortionless Enhancement by Polarization Transfer
HSQC	Heteronuclear Single-Quantum Coherence
HMBC	Heteronuclear Multiple-Bond Correlation
COSY	Correlated Spectroscopy
$\delta$	chemical shift
ppm	part per million
s	singlet
d	doublet
t	triplet
q	quartet
dd	doublet of doublet
dt	doublet of triplet
m	multiplet
TLC	Thin Layer Chromatography
MPLC	Medium Pressure Preparative Liquid Chromatography
$R_f$	Relative mobility
RP-18	Reversed-phase octadecyl silica gel
MTT	Methyl Thiazolytetrazolium
PBS	Phosphate-Buffered Saline
SDS	Sodium Dodecyl Sulfonate
IC <sub>50</sub>	Concentration giving 50% of maximal inhibition
MeOH	Methanol

---

CHCl <sub>3</sub>	Chloroform
EA	Ethyl acetate
PE	Petroleum ether
DMSO	Dimethylsulphoxide
mg	milligram
mL	milliliter
$\mu$ M	micromolar
$\mu$ g	Microgramme
r/min	Revolutions per minute
h	hour

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## 摘要

微生物次生代谢产物是药物的重要来源。近年来,新的微生物次生代谢产物的发现多数来自特异生境微生物和稀有微生物菌源的应用,如植物内生真菌、海洋微生物、土壤稀有放线菌等。这些微生物资源由于其生境特异性以及种属特异性,能够产生许多具有新颖化学结构和独特生物活性的次级代谢产物,为新药创制提供重要的先导化合物。

本论文对2株马尾松内生真菌拟茎点霉菌 *Phomopsis* sp. F00164 和小丛壳菌 *Glomerella* sp. F00244 以及1株土壤稀有放线马杜拉菌 *Actinomadura* sp. x11-7 的次级代谢产物进行了研究,共分离鉴定25个化合物,其中14个为新化合物。

从 *Phomopsis* sp. F00164 菌株的固体PDA培养基发酵粗提物中共分离鉴定了9个化合物,包括 alternariol (**F-4**), 2个 alternariol 衍生物: **F-2**、**F-3**; 4个 nonenolides 类化合物: **F-1**、**F-35**、**F-39**、**F-41**; 2个 phthalide 衍生物: **F-14**、**F-30**。其中 **F-1**、**F-30**、**F-35**、**F-39** 和 **F-41** 为新化合物。

从 *Glomerella* sp. F00244 菌株的固体PDA培养基发酵粗提物中共分离鉴定了6个化合物,包括2个 lanostane 型三萜: **H-3**、**H-8**; 1个 cerevisterol 衍生物 (**H-14**), 3个 ergosterol 类化合物: ergosterol (**H-1**), ergosterol peroxide (**H-29**) 及其D环衍生物 (**H-17**)。其中 **H-3**、**H-8** 和 **H-17** 为新化合物。

从 *Actinomadura* sp. x11-7 菌株的固体YMG培养基发酵粗提物中共分离鉴定了10个化合物,包括2个 pimarane 型二萜: **Eleven-1**、**Eleven-3**; 2个吡啶二聚体衍生物: **Eleven-5**、**Eleven-6**; 2个糖苷化合物: **Eleven-14**、**Eleven-17**; 1个 hydroxylactone 类化合物: **Eleven-19**; genistein (**Eleven-4**); N-acetyltyramine (**Eleven-28**)和 cybullol (**Eleven-30**)。其中 **Eleven-1**、**Eleven-3**、**Eleven-5**、**Eleven-6**、**Eleven-14** 和 **Eleven-19** 为新化合物。

对上述化合物的抗肿瘤活性和抗菌活性进行了初步测定。检测结果显示, **Eleven-10** 对人宫颈癌 Hela、人肝癌 HepG2 细胞株的  $IC_{50}$  分别为 11.59、4.70  $\mu\text{g/mL}$ ; **Eleven-4** 对 Hela、HepG2 细胞株的  $IC_{50}$  分别为 54.49、6.84  $\mu\text{g/mL}$ 。其他化合物在浓度为 10  $\mu\text{g/mL}$  时均未表现出明显的细胞毒活性。所有化合物均未表现出明显抗菌活性。

本论文的研究结果表明,植物内生菌和植物根际稀有放线菌中蕴藏着丰富的次级代谢产物,是寻找药物先导化合物的重要资源。

关键词:植物内生菌;稀有放线菌;次级代谢产物

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## Abstract

Bioactive natural products play a highly significant role in the drug discovery and development process, of which, microbial secondary metabolites take up a large proportion. In recent years, new microbial secondary metabolites were mostly searched from specific habitat microorganisms and rare microorganisms, such as plant endophytes and rare actinomycetes. Due to their specific habitat and genus, they can produce numerous metabolites with novel and varied skeletons and unique bioactivities. Furthermore, they can offer novel leads for new drugs.

In this thesis, secondary metabolites from two endophytic fungus and one rare actinomycete were observed for the discovery of new bioactive compounds. Totally, twenty-five compounds were isolated and elucidated, including fourteen new compounds.

Nine compounds were obtained from the agar cultures of the strain *Phomopsis* sp. F00164, including alternariol (**F-4**), two alternariol derivatives (**F-2**, **F-3**), four nonenolides (**F-1**, **F-35**, **F-39**, **F-41**) and two highly substituted phthalides (**F-14**, **F-30**). In which, **F-1**, **F-30**, **F-35**, **F-39**, **F-41** are novel.

Six compounds were isolated from the agar cultures of the strain *Glomerella* sp. F00244, including two lanostane-type triterpenes (**H-3**, **H-8**), a cerevisterol derivative (**H-14**), together with ergosterol (**H-1**), ergosterol peroxide (EP) (**H-29**) and a D-ring derivative of EP (**H-17**). In which, **H-3**, **H-8**, **H-17** are novel.

Ten compounds were isolated from the agar cultures of the strain *Actinomadura* sp. x11-7, including two pimarane-type diterpenes (**Eleven-1**, **Eleven-3**), two indole-3-carbinol dimer derivatives (**Eleven-5**, **Eleven-6**), one hydroxylactones (**Eleven-19**), genistein (**Eleven-4**), N-acetyltyramine (**Eleven-28**), cybullol (**Eleven-30**) and two glycosides derivatives (**Eleven-14**, **Eleven-17**). In which, **Eleven-1**, **Eleven-3**, **Eleven-5**, **Eleven-6**, **Eleven-14**, **Eleven-19** are novel.

The chemical structures of all these compounds were elucidated by spectroscopic and mass spectrometric analysis, including 1D-, 2D-NMR and ESI-MS.

None of these compounds showed obvious antimicrobial activity against *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus subtilis*, *Bacillus pumilus*, *Aspergillus niger* and *Candida Albicans* by inhibition zone assay.

**Eleven-10** was high cytotoxic in vitro towards Hela and HepG2 cell lines by

MTT assay, with IC<sub>50</sub> values of 11.59、4.70  $\mu\text{g/mL}$ , respectively. Meanwhile, **Eleven-4** also showed cytotoxic activity in vitro against Hela and HepG2 cell line with IC<sub>50</sub> values of 54.49、6.84  $\mu\text{g/mL}$ . Other compounds didn't show obvious cytotoxic activity against either of two cell lines.

Our results indicated that plant endophytes and rare actinomycetes can produce novel metabolites. They are important sources of searching leads for new drugs.

**Key words:** Plant endophytes; Rare actinomycetes; Secondary metabolites

## 第一章 前言

天然产物是新药创制的重要来源，当前所使用的药物中，80%为天然产物或其结构类似物<sup>[1]</sup>。虽然组合化学技术在药物发展进程中占据主要地位（图 1.1），但值得注意的是，当前的趋势直指运用多样性导向的合成技术建立复杂天然产物类似物库<sup>[2]</sup>，这就要求必须将天然产物与组合化学技术相结合。天然产物在新药创制中是必不可少的，正如 Danishefsky 形象地评论，“许多药物公司停止天然产物业务的决定是愚蠢的，天然产物中存在非常重要的教导，是值得我们永恒思考的智慧和精华。”<sup>[3]</sup>

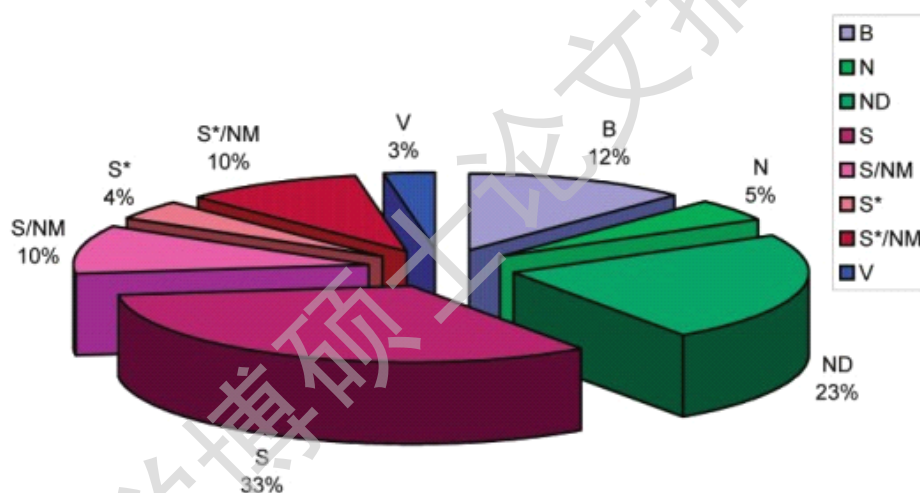


图 1.1 1981-2002 期间所有新药来源分类 (N=1031)

**Figure 1.1 All new chemical entities, 1981-2002, by source (N=1031) (From Newman, 2003)**

(“B”: Biological; usually a large (> 45 residues) peptide or protein either isolated from an organism/cell line or produced by biotechnological means in a surrogate host. “N”: Natural product. “ND”: Derived from a natural product and is usually a semisynthetic modification. “S”: Totally synthetic drug, often found by random screening/modification of an existing agent. “S\*”: Made by total synthesis, but the pharmacophore is/was from a natural product. “V”: Vaccine. “NM”: Natural Product Mimic. )

制药研究的大规模起始于青霉素的发现，通过广泛筛选具有抗菌活性的微



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